UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
4301 JONES BRIDGE ROAD
BETHESDA, MARYLAND 20814-4712
www.usuhs.mil

APPROVAL SHEET

Title of Thesis: "Pharmacological Treatment of Glutamate Excitotoxicity Following Traumatic Brain Injury"

Name of Candidate: Michael Doh
Molecular & Cell Biology Graduate Program
Master of Science
January 14, 2009

Thesis and Abstract Approved:

Joseph McCabe
Joseph McCabe, PhD
Department of Anatomy, Physiology & Genetics
Thesis Advisor

01-14-2009
Date

Aviya Symes, PhD
Department of Pharmacology
Committee Member

01/14/2009
Date

Tao-John Wu, PhD
Department of Obstetrics & Gynecology
Committee Member

1/14/2009
Date

Learning to Care for Those in Harm's Way

Report Documentation Page

Form Approved
OMB No. 0704-0188

Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.

1. REPORT DATE
14 JAN 2009

2. REPORT TYPE

3. DATES COVERED
00-00-2009 to 00-00-2009

4. TITLE AND SUBTITLE
Pharmacological Treatment Of Glutamate Excitotoxicity Following Traumatic Brain Injury

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Uniformed Services University of the Health Sciences,4301 Jones Bridge Rd,Bethesda,MD,20814

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Traumatic Brain Injury (TBI) is a major public health concern. A TBI is defined as a blow to the head that results in the disruption of normal brain function. Patients who suffer a TBI exhibit a wide variety of cognitive, somatic and psychological symptoms that can severely diminish the individual?s quality of life. Despite continued research, no therapy has proven effective for the treatment of TBI.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**Same as Report (SAR)**	**52**	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std Z39-18

The author hereby certifies that the use of any copyrighted material in the thesis manuscript entitled:

"Pharmacological Treatment of Glutamate Excitotoxicity Following Traumatic Brain Injury"

Is appropriately acknowledged and, beyond brief excerpts, is with permission of the copyright owner.

Michael Doh
Department of Molecular and Cell Biology
Uniformed Services University

ABSTRACT

Title of Thesis: “Pharmacological Treatment of Glutamate Excitotoxicity Following Traumatic Brain Injury”

Michael Doh, Master of Science, 2009

Thesis directed by: Joseph McCabe, PhD
Thesis Advisor
Department of Anatomy, Physiology and Genetics

Traumatic Brain Injury (TBI) is a major public health concern. A TBI is defined as a blow to the head that results in the disruption of normal brain function. Patients who suffer a TBI exhibit a wide variety of cognitive, somatic and psychological symptoms that can severely diminish the individual’s quality of life. Despite continued research, no therapy has proven effective for the treatment of TBI.

The pathophysiology of the injured brain is complex. Cell death following TBI can result from primary injuries, caused by mechanical forces associated with the insult, and secondary injuries, caused by the disruption of normal cellular function in the injured brain. Secondary injuries include brain swelling, hypoxia, oxidative stress, and glutamate excitotoxicity.

The following will serve to discuss the basic phenomenology of TBI, focus on the role of glutamatergic neurotransmission and its role in TBI pathology, and analyze current pharmacological treatment strategies.

PHARMACOLOGICAL TREATMENT OF GLUTAMATE EXCITOTOXICITY FOLLOWING TRAUMATIC BRAIN INJURY

By

Michael Doh

Thesis submitted to the Faculty of the
Molecular and Cell Biology Graduate Program
Uniformed Services University of the Health Sciences
In partial fulfillment of the requirements for the degree of
Master of Science 2009

TABLE OF CONTENTS

Approval Sheet i

Copyright Statement ii

Abstract. iii

Title Page. iv

Table of Contents. v

List of Figures. vi

Traumatic Brain Injury. 1
- Incidence and Causes 1
- Signs and Symptoms 2
- Classification. 4
- Pathophysiology. 5

Glutamate Excitotoxicity Following Traumatic Brain Injury. 8
- Glutamate Synapse. 9
- Glutamate Excitotoxicity 11
- Sources of Excitotoxicity in TBI 13
- Consequences of Excitotoxicity 14
- Correlation of Excitotoxicity with Symptoms of TBI 16

Pharmacological Strategies for the Treatment of Excitotoxicity in TBI 18
- Glutamate Release. 19
- Glutamate Receptor Antagonists. 20
- Ion Homeostasis. 21
- Blood Glutamate Scavenging. 22
- Glutamate Transport. 23

Materials and Methods. 25

Results. 27

Discussion 29

Conclusion. 29

References. 37

FIGURES

Figure 1: GLT-1 protein expression following TBI. 31

Figure 2: GLT-1 mRNA expression following TBI. 32

Figure 3: GLT-1 protein expression following ceftriaxone treatment. 33

Figure 4: GLT-1 mRNA expression following ceftriaxone treatment. 34

Figure 5: Effect of ceftriaxone on TBI, cortex. 35

Figure 6: Effect of ceftriaxone on TBI, hippocampus. 36

Traumatic Brain Injury: Incidence and Causes

Traumatic brain injury affects millions of people worldwide. In 2003, an estimated 1.5 million patients suffered a TBI in the United States alone (Rutland-Brown 06). Nearly eighty percent of these patients visited the emergency department, with 300,000 requiring hospitalization. Approximately 50,000 people die each year in the U.S. as a result of TBI, while 80,000 will suffer a chronic neurological disability following injury (Rao 00). The economic cost of TBI in the U.S. is in excess of $35 billion dollars annually, including costs for medical care and lost productivity due to death and disability (Thurman 99).

The incidence of TBI varies with a number of factors, including age, gender and socio-economic status. Children under the age of four and adults over seventy-five are most at risk for TBI (Rutland-Brown 06). Compared to females, males are two times as likely to suffer a TBI and four times as likely to die as a result of their injury. The incidence of TBI is also significantly higher in economically-disadvantaged populations (Kraus 93).

The leading causes of TBI include motor vehicle accidents, falls, sport-related injuries, and assaults (Langlois 04). Motor vehicle accidents represent twenty percent of TBI cases and over thirty percent of TBI-related fatalities. Adolescents between the ages of 15 and 19 are most at risk for sustaining a TBI in a traffic incident. Injuries resulting from falls comprise nearly thirty percent of TBI cases but only ten percent of TBI fatalities (Thurman 99). The majority of TBIs in persons over 75 are caused by falls. Firearms are the most common cause of TBI fatalities (39%), as only nine percent of cases will survive (Rutland-Brown 06). In infants, child abuse is the leading cause of TBI (64%). Combat-related TBI is becoming more prevalent due to recent military action in Iraq and Afghanistan (Warden 06). An increasing number of soldiers have been stricken with TBI related symptoms after surviving blast-related injuries.

Traumatic brain injury affects all types of populations, and depending on the severity of the injury, individuals can experience long-term, permanent neurological disabilities. Because there is no effective therapy for the treatment of TBI, patients will suffer a lifetime of physical, psychological and economic difficulties. Therefore, continued research is essential for developing new pharmacological strategies to improve the quality of life for TBI patients.

Traumatic Brain Injury: Signs and Symptoms

Individuals suffering a TBI can exhibit a wide variety of symptoms and complications as a result of their injuries. These symptoms can be classified as somatic, psychological or cognitive. Somatic symptoms are those that are related to physical characteristics, including pain, sensation, and movement. The most common symptom of head trauma is headaches, reported in 30-90% of patients (Solomon 01). The occurrence of headaches can persist for an extended duration of time, as 8-32% of patients complained of increased headaches one year after injury (Weight 98). The second most common complaint of TBI patients is dizziness, which occurs in 50% of cases, and 19-25% of individuals still experience dizziness after one year (Brown 94), The incidence is higher in older patients.

TBI also can affect sensory perception. Blurred vision affects 15% of patients (Hall 05), while ten percent experience increased sensitivity to light and sound. Additionally, five percent of cases involved decreased smell and taste.

TBI can also have adverse effects on movement, causing a decrease in coordination with increased feelings of fatigue and lethargy. In cases of more severe injury, patients can suffer from post-traumatic seizures (Agrawal 06). TBI has also been identified as a risk factor for the development of Parkinson’s disease (PD) (Bower 03). PD is a chronic and progressive movement disorder characterized by tremors, stiffness, slowed movement (bradykinesia), and stooped posture.

TBI can also cause a wide variety of psychological symptoms, including changes in mood or personality, anxiety or depression. Nearly 50% of individuals report some type of psychiatric difficulty within three months (Rao 02), with 25% classified as clinically depressed, and a small number (0.7-9.8%) suffering from schizophrenia-like psychosis. TBI patients are also a higher risk for developing psychological problem many years post-injury.

Cognitive symptoms of brain injury include problems with memory/learning, attention/concentration, and language/communication. Memory impairment is seen in 20-79% of head-injured patients, with either the loss of memories (retrograde amnesia) or the inability to form memories (anterograde amnesia) (Hall 05). Memory deficits persist after one year in 4-25% of cases. In addition, studies have shown that a TBI may contribute to the development of Alzheimer disease (Szczygielski 05), a neurodegenerative disorder characterized by dementia and memory loss.

Traumatic Brain Injury: Classification

A traumatic brain injury can be classified as mild, moderate and severe. The Glasgow Coma Score (GCS) is a standard measure of identifying the severity of the injury. Eye, vocal and motor responses are tested and the patient is scored from three (most severe) to fifteen (least severe) (Teasdale 74). Other measures of severity include duration of post-traumatic amnesia (PTA), and loss of consciousness (LOC). Mild head injuries are defined as those with a GCS of 13-15, with PTA of less than 1h, and LOC of less than 30min. Moderate injuries have a GCS of 9-12, with PTA of 30min-24h, and LOC of 1-24h. The most severe brain injuries have a GCS of 3-8, with amnesia and loss of consciousness lasting for longer than 24 hours (Rao 00).

TBI can also be classified based on the nature of the injury. Open-head, or penetrating injuries occur when an object pierces the skull (Blissitt 06). Gunshots and knife wounds are examples of open-head injuries, and are usually considered the most severe. These types of injuries can result in skull fractures and cerebral lacerations which cause damaged blood vessels, disruption of the blood-brain barrier and meninges, brain tissue displacement, the rupturing of cellular membranes, and neuronal cell death (Roth 00). Penetrating injuries are considered focal injuries, where the injury is localized to one region of the brain.

Closed-head, or blunt injuries occur when the skull is not penetrated. These are generally considered more mild forms of TBI, and are caused by motor vehicle accidents and falls. Closed-head injuries can result in cerebral contusion and diffuse axonal injury (Khoshyomn 04). Cerebral contusions are bruises on the surface of the brain caused by the brain moving within the skull. Contusions occur in 20-30% of injuries and are caused by a blow to the head or acceleration/deceleration forces. Cerebral contusions can lead to swelling of the brain and an increase in intracranial pressure (ICP). Diffuse axonal injury (DAI) is the disruption of axons caused by shearing forces as tissues and cells slide against one another (Roth 00). DAI is an example of diffuse head injury that occurs over a widespread area and is typical of acceleration-deceleration injuries.

Traumatic Brain Injury: Pathophysiology

Brain damage caused by TBI can be characterized as primary injury, directly resulting from the mechanical forces from the insult, and secondary injury, cell death resulting from the disruption of normal brain function. Skull fractures, cerebral lacerations, contusions and diffuse axonal injury are considered primary injuries. Primary injuries cause physical damage to cell membranes and brain structures, resulting in non-specific cell death. Another type of primary injury is hemorrhaging, which can be classified as intracerebral, subdural, subarachnoid or epidural, depending on the site of the bleeding (Roth 00). Cerebral bleeding can be caused by open- and closed-head injuries and is associated with both focal and diffuse injury. This bleeding causes disruption in the delivery of blood to brain tissues and cells. Cerebral contusions and bleeding can also lead to an increase in intracranial pressure (ICP), which can result in brain herniation, or the squeezing of brain tissue within the skull. Because the primary injury occurs immediately at the point of impact, it is not preventable by therapeutic intervention and for the most part, the injuries are irreversible. However, the characterization of the consequences of primary injury contributes to the understanding of the processes that lead to subsequent secondary cell death.

The major consequences of primary injuries include the alteration of cerebral blood flow, affecting oxygen availability, the disturbance of the blood brain barrier and the cell membrane disruption (Werner 99). These lead to secondary injuries in the hours to days following the initial insult. Secondary injuries result from the disruption of normal brain function and greatly contribute to the brain damage associated with TBI. A significant percentage of TBI fatalities occur in the days to weeks post-injury, and the health of 40% of injured individuals deteriorates after hospitalization (Narayan 02).

Alterations in cerebral blood flow (CBF) is an example of secondary injury. Under normal conditions CBF is tightly regulated based on metabolic demand. TBI can result in hyperperfusion, or an increase in CBF (Kelly 97), causing a dangerous increase in ICP. Hypoperfusion, or a decrease in CBF leads to inadequate blood supply causing ischemia. Hypoxia, or a decrease in oxygen supply, decreases the ability of neurons to produce ATP via normal metabolism. This results in the inability to maintain membrane potential by ATP pumps, mitochondrial dysfunction, and the promotion of apoptosis, leading to cell death. Coles, et al. (2004) used $^{15}O_2$ positron emission CT to assess CBF following TBI. They found that decreased CBF indicated the presence of ischemia and correlated with poor outcome.

Brain swelling, or edema, is another form of secondary brain injury. Vasogenic edema is caused by the disruption of the endothelial layer of blood vessels resulting in the unregulated transfer of ions and proteins across the blood brain barrier

(Unterberg 04). Cytotoxic edema is caused by the increased ion permeability of cell membranes under hypoxic conditions (Stiefel 05). Both types of edema contribute to increased ICP.

Inflammation also contributes to cell death following TBI. TBI activates the release of proinflammatory cytokines, prostagladins, free radicals and complement (Lucas 06) which mobilize immune and glial cells. Damaged cells and adjacent tissue are eliminated by the actions of proinflammatory enzymes, cytokines and neurotoxic mediators. Inflammation promotes the release of potentially toxic factors into the extracellular space and the clearance of damaged but still viable cells.

Oxidative stress can also have pathological consequences following brain injury. Reactive oxygen species (oxygen radicals, superoxides, hydrogen peroxide, nitric oxide) are formed by metabolism and are involved in normal cell functions (Werner 99). An increase in ROS following TBI leads to protein and lipid oxidation, damage to DNA, and mitochondrial dysfunction.

Glutamate excitotoxicity, another form of secondary injury, is defined as cell damage resulting from the overactivation of glutamate receptors. It is caused by an excess of glutamate in the extracellular space (Yi 06). The pathological increase in glutamate results from both decreases in ATP production and disruption of the blood-brain barrier and cellular membranes. The consequences of excitotoxicity include cell death by apoptosis, mitochondrial dysfunction, increased oxidative stress and inflammation, and edema. Because excitotoxicity is related to other facets of secondary injury, it is an attractive target for therapeutic intervention.

Glutamate Excitotoxicity Following Traumatic Brain Injury

The amino acid glutamate functions as a neurotransmitter in the mammalian central nervous system. Glutamate, and to a lesser extent aspartate, directs most of the excitatory synaptic transmission in the CNS (Doble 99). Excitatory neurotransmission results in the depolarization of the postsynaptic, or target, cell promoting the propagation of action potentials. On the other hand, inhibitory neurotransmission mediated by GABA, causes the hyperpolarization of the postsynaptic cell membrane, dampening the generation of action potentials.

Glutamate is used as a neurotransmitter throughout the cortex, hippocampus, cerebellum and other brain regions (Cotman 87), accounting for one third of rapid excitatory synapses in the CNS. Among its many roles in the brain, glutamate signaling has been implicated in synaptic plasticity. Synaptic plasticity is defined as activity dependent modification of synaptic efficiency (Albensi 03). Bliss, et al. (1973) found that stimulation of the perforant path in rabbits led to an increase in synaptic efficiency of the stimulated cells. This phenomenon is known as long-term potentiation (LTP). Long-term depression (LTD) relates to the decrease in synaptic efficiency following either weak or strong activation. LTP and LDP are believed to be the basis for learning and memory (Izquierdo 97).

Glutamate signaling is involved in a wide variety of somatic, psychological and cognitive processes. Alterations in glutamate function have been associated with many of the symptoms of TBI including headaches, depression, problems with learning and memory, and the increased risk for the development of neurological disorders. Therefore, glutamate signaling is an attractive target for developing therapeutic strategies for the treatment of brain injury.

Glutamate Synapse

Glutamate is stored in synaptic vesicles along the presynaptic membrane at a concentration of 20mM. The resting potential of an axonal membrane is maintained at -70mV by the transport of sodium and potassium ions through pumps in an ATP-dependent manner. As an action potential travels along the axon, the membrane potential is briefly depolarized to 40mV (Doble 99). When the action potential reaches the synapse, glutamate is released from the vesicles into the synaptic cleft. The concentration of glutamate in the synaptic cleft rises transiently to 1-2mM, where it binds to and activates receptors on the postsynaptic membrane. This leads to a number of changes in the postsynaptic cell including alterations in ion permeability, enzyme activity and gene expression.

Glutamate receptors are classified as ionotropic receptors and metabotropic receptors. An ionotropic glutamate receptor is comprised of an intrinsic transmembrane ion channel which opens and closes upon the binding of glutamate. There are three functional classes of ionotropic glutamate receptors (Foster 84), named for their selective agonists: N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), and kainate (KA). Ionotropic glutamate receptors are multimeric assemblies of individual subunits. This allows for a wide variety of possible receptors. Splice variants and RNA editing of the individual subunits also contributes to the vast heterogeneity of ionotropic receptor composition and functionality (Zukin 95).

Metabotropic glutamate receptors are linked to enzymes by G proteins and are classified according to selective agonists and signal transduction pathways (Toms 96). 3,5-dihydroxyphenylglycine selectively activate Group I metabotropic receptors, which activate phospholipase C (PI-PLC) and promote the release of Ca^{2+} ions from cytoplasmic stores. Group II receptors are selectively activated by 2R,4R-4-aminopyrrolidine-2-4-dicarboxylate (APDC) and inhibits the action of adenylyl cyclase. Group III receptors also inhibit adenylyl cyclase and are selectively activated by L-amino-4-phosphonobutyrate (L-AP-4).

Following the release of glutamate from the presynaptic neuron and the activation of receptors on the postsynaptic membrane, the signal is terminated by the removal of glutamate from the synaptic cleft via the action of Na^{+}-dependent glutamate transporters. Five subtypes of glutamate transporters have been cloned to date, excitatory amino acid transporters 1-5 (EAAT1-5) (Danbolt 01). EAAT1, also known as the glutamate-aspartate transporter (GLAST), is predominantly expressed in glial cells in the cerebellum and is responsible for the uptake of glutamate from the extrasynaptic space (Rothstein 94). EAAT2, or glutamate trasporter-1 (GLT-1) is expressed in glial cells throughout the brain (Danbolt 92) and is involved in the transport of glutamate from the synaptic cleft. EAAT3-5 are mostly expressed in neuronal cells (Dehnes 98, Arriza 97).

The amino acid glutamate does not cross the blood-brain barrier; its synthesis and metabolism occur in distinct brain compartments, neurons and glial cells (Shen

08). After glutamate is transported into glial cells from the synaptic cleft, it is converted into glutamine by the enzyme glutamine synthetase. The glutamine is then transported back into the neuron where it is deaminated by phosphate-activated glutaminase (PAG), once again forming glutamate. Glutamate can also be synthesized as a product of aerobic metabolism and the Krebs cycle.

Glutamate signaling and metabolism is a highly complicated process that is dependent on proper energy metabolism, maintenance of membrane potential, and compartmentalization of metabolic enzymes. The disruption of these biological processes under pathological conditions can have numerous deleterious consequences.

Glutamate Excitotoxicity

In 1957, Lucas and Newhouse first described the neurotoxic potential of the amino acid glutamate. They found that a single intraperitoneal injection of free glutamic acid generated severe retinal lesions in immature and, to a lesser extent, adult mice. Further studies described similar glutamate-induced retinal lesions in the rat and rabbit. In 1969, Olney found that subcutaneous injection of monosodium glutamate resulted in necrotic brain lesions in the hypothalamus of newborn mice, leading to a number of developmental abnormalities. These reports led to the introduction of the term “excitotoxicity,” describing cell damage induced by an excess of excitatory amino acids.

Since then, numerous studies have investigated the potential toxic effects of glutamate in the context of neurological disorders. Several studies have examined the levels of extracellular glutamate in experimental models of TBI. Using microdialysis, Faden et al (1989) analyzed the concentrations of excitatory amino acids in the rat brain using the fluid percussion injury model of TBI. They found that extracellular glutamate was significantly increased at the site of injury; 282% in moderately injured animals and 940% in severely injured animals. The elevations in glutamate sharply peaked at 10 minutes post-injury, and persisted for more than one hour in the severe injury. The administration of dextrorphan (10mg/kg, i.v., 30 min post-injury), a noncompetitive NMDA antagonist, improved neurological outcome, suggesting that glutamate is involved in TBI induced brain damage.

Glutamate levels have also been studied in human TBI patients. Baker, et al. (1993) measured glutamate in the cerebrospinal fluid of twelve head injured patients. They determined that the peak glutamate concentrations were significantly higher (14-474mM) in the injured patients when compared to control (4.9-17mM). Numerous other studies have confirmed that glutamate levels are increased in the human brain following injury (Ruppel 01, Tolias 02, Bullock 89). The elevated levels of glutamate strongly correlate with focal contusions, sustained high ICP and poor outcome. The increase in glutamate seems to peak between 12 and 72 hours following injury, and remains slightly but persistently elevated for as long as eight days.

The temporal changes in glutamate concentration following injury are an important consideration when devising a treatment strategy. The acute increase in glutamate may occur within too narrow a time frame for therapeutic treatment (Ikonomidou 02). Additionally, because glutamate serves many biological roles, its sustained elevation may be involved in the body’s response to the injury. Finally, cell death following injury can result from “slow excitotoxicity” (Albin 92), in which cells are rendered vulnerable to physiologic concentrations of glutamate due to pathological conditions.

Due to the evidence that glutamate is involved in TBI-induced brain damage, there has been much research into the possible sources of excess glutamate in TBI, as well as the consequences of excitotoxicity.

Sources of Excitotoxicity in TBI

Multiple sources of extracellular glutamate increase following TBI have been proposed. The primary injuries associated with TBI can lead to a disruption of the blood-brain barrier. Koizumi, et al. (1997) examined the diffusion of blood glutamate in a weight-drop model of TBI in the rat. Immediately following injury, they injected ^{14}C-labeled glutamate into the femoral vein. Autoradiography revealed a significant extravasation of labeled glutamate from the vascular component into the cortex at the site of impact, indicating that extracellular glutamate is increased by the disruption of the BBB. Studies have also shown that glutamate is released non-specifically from damaged membranes. Bullock, et al. (1989) demonstrated that the increase in glutamate following TBI was correlated with an increase in structural amino acids. This suggested that glutamate can be released from injury-induced membrane micropores along with other amino acids, rather than from presynaptic vesicles.

Other studies have shown that increased release from presynaptic neurons can also contribute to excitotoxicity. Yi, et al. (2006) found that there is an increased expression of the protein complexin II following injury. This protein is involved in the proper maintenance of neurotransmitter release, and its increased expression may represent a disruption of normal synaptic function.

Another potential source of excitotoxicity is glutamate transport. Under normal conditions, glutamate is removed from the synapse by the action of the astrocytic transporter GLT-1. Rao, et al. (1998) showed that GLT-1 expression was significantly reduced (38-47%) in the rat brain 24 hours after fluid percussion injury. The resulting decrease in transport leads to an excess of glutamate in the synaptic cleft. Additionally, it has been proposed that the action of glutamate transporters is reversed at the injury site where the damage is most severe (Phillis 00). This would cause a spilling of glutamate from astrocytes into the extracellular space.

Another major contributor to excitotoxicity is energy deprivation. Under ischemic conditions, a decrease in oxygen availability leads to a decrease in ATP production. ATP is required to maintain neuronal membrane potential, and a decrease in energy results in the depolarization of the membrane. This leads to the improper generation and propagation of action potentials, causing a “spreading depression” (von Baumgarten 08) or a wave of cortical depolarization which can affect cells far from the site of injury.

Consequences of Excitotoxicity

In addition to the research on the sources of excitotoxicity in TBI, there have been numerous studies on the molecular consequences of excess glutamate, and the mechanisms of glutamate mediated neurodegeneration. Studies have shown that excitotoxicity is mediated mainly by alterations in the concentrations of Na^+ and Ca^{2+}

following overactivation of ionotropic glutamate receptors. Rothman (1985) demonstrated that glutamate exposure of cultured hippocampal neurons resulted in irreversible toxic swelling that was dependent on Na^+ influx and independent of Ca^{2+}. The removal of Na^+ prevented neuronal swelling but did not prevent delayed neuronal cell death. This long-term neuronal degeneration was attenuated only by the removal of Ca^{2+} from the extracellular media. Thus, he concluded that excitotoxicity is comprised of an acute Na^+-dependent toxicity, followed by a delayed Ca^{2+}-dependent toxicity.

Excessive Ca^{2+} can have many deleterious consequences. The release of glutamate from neurotransmitter vesicles is triggered by the influx of Ca^{2+} into the presynaptic neuron upon the generation of an action potential. Therefore, Ca^{2+} overloading results in the improper generation and propagation of excitatory signals. Pathological increases in Ca^{2+} can also damage mitochondria which maintain calcium homeostasis under normal conditions (Atlante 99). Excess Ca^{2+} leads to a release of cytochrome c from the mitochondria which activates caspase-9 and leads to cell death by apoptosis. Mitochondrial damage can also result in reduced ATP production and the toxic release of ROS.

Elevated concentrations of Ca^{2+} can also activate the cystein protease calpain, which is involved in the proteolysis of the cytoskeletal protein alpha spectrin after TBI (Pike 1998). Yakovlev, et al. (2001) found that following a controlled cortical impact injury in the mouse, Ca^{2+} dependent endonuclease activation resulted in DNA fragmentation. Additionally, excitotoxic Ca^{2+} overload results in activation of phospholipase A2 and nitric oxide synthase, increasing oxidative stress.

Although excitotoxicity is thought to be mediated predominantly by ionotropic glutamate receptors, activation of the metabotropic glutamate receptors is believed to be involved in modulating excitotoxicity. In vitro studies showed that activation of group I receptors exacerbated cell death induced by mechanical injury (Muhkin 96), whereas group II receptor agonists proved neuroprotective in an animal model (Zwienenber 01). Therefore, both ionotropic and metabotropic glutamate receptors represent possible therapeutic targets for the treatment of TBI.

Correlation of Excitotoxicity with Symptoms of TBI

Brain damage following TBI is multifaceted. Many different factors can contribute to the neuronal death and dysfunction following injury. However, numerous reports suggest that glutamate excitotoxicity is a major contributing factor to the symptoms associated with TBI.

Patients who suffer a TBI are at risk for developing post traumatic epilepsy (PTE). Penetrating injuries are associated with a 50% increased risk for PTE, while closed head-injuries are associated with a 30% increased risk (Agrawal 06). Various factors are involved in the pathogenesis of PTE. Disruption of the blood brain barrier leads to the extravasation of blood into the extracellular space in the brain.   This leads to an increase in iron concentration and the hyperproduction of hydroxyl radicals by an iron-catalyzed reaction (Willmore 78) which contributes to the development of seizures. Overactivation of NMDA receptors not only increases oxidative stress but is also associated with the formation of a chronic epileptogenic focus in iron-induced seizures (Janjua 90). Although excitotoxicity is not the only contributing factor to the development of post-traumatic epilepsy, its reduction could potentially have a beneficial effect in treating seizures following injury.

The most common symptom of TBI is headaches. Studies have shown that migraine headaches may, in part, be caused by glutamate excitotoxicity. In fact, NMDA receptor antagonists have been proposed as a treatment for migraines (Peeters 07). In the context of TBI, excitotoxicity can contribute to and result from spreading depression (Rogatsky 03). Studies have also shown that cortical depolarization caused by spreading depression activates the trigeminal nociceptive pathway, initiating the headache (Bolay 02). Therefore, reducing excitotoxicity following TBI may have a therapeutic effect on post-traumatic migraines.

Excitotoxicity has also been implicated in the pathology of depression. Although previous studies have implicated serotonin, norepinephrine and dopamine as mediators of depression, recent evidence suggests that glutamatergic dysfunction may also be involved (Chourbaji 08). Sanacora, et al. (2000) found that the levels of glutamate in the brain was significantly higher in depressed patients as opposed to control. Interestingly, a clinical trial found that riluzole, a glutamate-reducing drug, significantly improve outcome when administered to patients diagnose with major depressive disorder (Zarate 04). Again, glutamatergic dysfunction is not solely responsible for the psychological symptoms associated with TBI, but targeting excitotoxicity may have a beneficial effect.

Finally, excitotoxicity has been associated with problems with memory and learning. TBI is a risk factor for the development of Alzheimer disease (AD). Lesne, et al. (2005) demonstrated that NMDA receptor activation promotes the production of the toxic peptide beta-amyloid Aβ, which accumulates in the brain of AD patients. Smith, et al. (2003) found that amyloid immunoreactivity was significantly increased in swollen axons in 9 of 12 patients who had died following a head injury. Olsson, et al. (2004) measured the concentration of Aβ(1-42) in the ventricular CSF of 41 severe head injured patients using ELISA. They found that the concentration of Aβ(1-42) in the VCSF was significantly increased (573%) 1 day after injury, peaked (1173%) at 5-6 days, and remained elevated (855%) 7-10 days post injury. *In vitro* studies have shown that Aβ alters the expression of many proteins at the glutamate synapse: NMDA receptors (Goto 06), AMPA receptors (Almeida 05), and PSD-95, a scaffolding protein (Roselli 05). In animal models of brain injury, the expression of these proteins is decreased (Colbourne 03, Osteen 04, Gascon 08). Therefore, overactivation of NMDA receptors following TBI increases amyloid deposition which in turn alters the expression of glutamatergic proteins, effecting synaptic efficiency. This may interfere with synaptic plasticity and may contribute to the memory and learning deficits associated with TBI.

Because of the many deleterious consequences of excitotoxicity following TBI, there has been much research into developing therapeutic strategies to combat glutamate mediated cell damage.

Pharmacological Strategies for the Treatment of Excitotoxicity in TBI

There is a wealth of evidence implicating glutamate excitotoxicity as a major contributing factor to brain damage. Based on these findings, a number of pharmacological strategies have been proposed. These treatment strategies target various facets of TBI-induced excitotoxicity including glutamate release, blood glutamate, receptor activation, transport, disruption of ion homeostasis, and downstream effectors.

Glutamate release

The prevention of glutamate release has been proposed as a potential therapeutic strategy for TBI. In vitro studies have demonstrated that cannabinoids inhibit glutamate release in rat hippocampal neurons (Shen 96). The endocannabinoid 2-arachidonoyl glycerol was found to be neuroprotective in a mouse model of closed head injury (Panikashvili 06), though its effect may be mediated in part by the inhibition of inflammatory cytokines. Despite the evidence that cannabinoid treatment could be therapeutic, clinical trials with Dexanabinol (HU-211) have proven ineffective for improving outcome following TBI (Maas 06). In a placebo-controlled phase III clinical trial, 846 patients were given an injection of dexanabinol (150mg iv) or placebo within 6 hours of injury. Glasgow outcome score was assessed 6 months following injury. They found no significant improvement in outcome between the drug treated and control groups

The sodium channel blocker, Riluzole, is another glutamate release inhibitor that has proven neuroprotective in animal models of TBI (Wahl 97, Zhang 98). Wahl showed that Riluzole treatment improved lesion size and sensorimotor functions of rats subjected to FPI. It is currently being evaluated in a phase III clinical trial for TBI in Europe (Marklund 06)

Glutamate Receptor Antagonists

The use of glutamate receptor antagonists to combat glutamate excitotoxicity has been extensively described. Because excitotoxicity is largely mediated by the overactivation of NMDA receptors, the selective blockage of these receptors is proposed to be neuroprotective. In fact, clinical trials using NMDA receptor antagonists have shown some effectiveness in treating neurological disorders involving excitotoxicity including Alzheimer disease (Reisberg 03), Huntington's disease (Lucetti 03) and epilepsy (Richens 00).

There have been several studies that have examined the potential neuroprotective effect of glutamate receptor antagonists in animal models of TBI. Kroppenstedt, et al. (1998) injected rats with the NMDA receptor antagonist aptiganel, or Cerestat, (2mg/kg b.w.) fifteen minutes after injury in a controlled cortical impact model. They found that the drug significantly reduced lesion volume (13.6%) and hemispheric swelling (31.5%) associated with TBI. In a subsequent study, Rao, et al. (2001) found that treatment with the non-competitive NMDA blocker, memantine, almost completely prevented TBI-induced neuronal cell loss in the CA2 and CA3 regions of the hippocampus. Similar neuroprotective effects have been described for other NMDA channel blockers, dextromethorphan, dextrorphan, ketamine and MK-801 (McIntosh 98).

In 1998, Morris, et al. conducted a phase III clinical trial of Selfotel (CGS 19755) for treatment of severe head injury. A total of 693 patients with GCS of 4-8 were enrolled in two multicenter double blind studies. Patients were injected with either a placebo or Selfotel (5mg/kg i.v) once a day for four days and the primary outcome of improved GCS was assessed at six months. The trial was stopped prematurely due to the number of adverse events. An analysis of the results showed no significant improvement in GCS in Selfotel-treated patients as compared to the placebo.

In a subsequent study, Yurkewicz, et al. (2005) studied the effect the NMDA receptor antagonist, traxoprodil, following TBI. A total of 404 patients were given a 72h infusion of the NMDA receptor antagonist traxoprodil (0.75mg/kg/h) within 8 hours of the injury. Although the drug was well tolerated, there was no significant improvement in GCS assessed at 1, 3, and 6 months post injury.

Although glutamate receptor antagonists have proven neuroprotective in animal models of excitotoxicity, clinical trials have been largely unsuccessful. Several factors may contribute to the poor response of these drugs (Ikonomidou 02). The use of receptor antagonists will block the activation of cells distal to the site of injury, interfering with physiological functions of uninjured cells. Activation of glutamate receptors not only promotes excitotoxicity, but is also involved in normal cell signaling. Therefore, blocking glutamate receptors following an injury may inhibit the body’s response to injury. Because of the ineffectiveness of glutamate receptor antagonists, other therapeutic strategies have been considered.

Ion homeostasis

As previously stated, Ca^{2+} overloading caused by NMDA receptor overactivation mediates many of the toxic effects of glutamate. The N-type calcium channel blocker, Ziconotide, is effective in animal models (Berman 00) but has not been used in clinical trials due to hypotensive side effects.

The L-type voltage-sensitive calcium channel blocker, nimodipine, has proven to be neuroprotective in experimental models (Geddes-Klein 06), and several clinical trials have tested the efficacy of nimodipine treatment in severely head-injured patients (Bailey 91, Teasdale 92, Kakarieka 94, Murray 96). In the first two trials, Head Injury Trial (HIT) I and II, nimodipine treatment showed no significant improvement in outcome in patient pools of 351 and 852. In a third trial with 123 patients, nimodipine significantly improved outcome following TBI with subarachnoid bleeding. However, a final worldwide multicenter trial with 591 participants failed to confirm a significant therapeutic effect of nimodipine following TBI.

Blood glutamate scavenging

Recent evidence suggests that glutamate is released into the brain from the vascular component following disruption of the blood brain barrier. Thus, compounds that can remove or “scavenge” glutamate from the blood could potentially reduce excitotoxicity in the brain. Oxaloacetate (OxAc) is an intermediate of the citric acid cycle and gluconeogenesis. OxAc and glutamate are reversibly converted to aspartate and α-ketoglutarate by the action of aspartate transaminase. An increase in the substrate OxAc decreases blood glutamate and creates a gradient which can lead to an efflux of glutamate from the brain into the blood, effectively reducing excitotoxicity.

In 2006, Zlotnik et al examined the neuroprotective potential of oxaloacetate (OxAc) in a rat model of closed head injury. Using a weight drop model of TBI, Zlotnik et al found that treatment with OxAc (1mmol/100g b.w., i.v.) significantly reduced blood glutamate levels (40%) and improved the neurological outcome of test animals. No neuroprotection was observed when OxAc was administered together with glutamate, and neurological recovery was enhanced by the coadministration of the enzyme (0.14nmol/100g b.w.). Subsequent studies (Zlotnik 08) showed that treatment with pyruvate had similar neuroprotective scavenging effects. Although these compounds have potential antioxidant and energetic effects, blood glutamate scavenging is an attractive new therapeutic approach that can combat excitotoxicity without the adverse side effects of other drug treatments. It may be particularly effective in alleviating the acute increase in glutamate immediately following injury.

Glutamate Transport

The modulation of glutamate transport has been proposed for the treatment of excitotoxicity associated with TBI. The glutamate transporter, GLT-1, removes glutamate from neuronal synapses (Rothstein 96) and its dysfunction has been linked to a number of neurological disorders, including amyotrophic lateral sclerosis (ALS), stroke, and epilepsy (Rothstein 95, Rao 01, Sepkuty 02). Thus, an increase in GLT-1 expression could potentially reduce excitotoxicity. In 2005, Rothstein, et al. used a luciferase reporter assay to screen 1,040 FDA-approved drugs for increased expression of GLT-1 in rat organotypic slice cultures. They found that β-lactam antibiotics significantly increased transcription at the human GLT-1 promoter. They next examined the neuroprotective effect of ceftriaxone in G93A SOD1 mice, a model of ALS. Ceftriaxone was chosen because it has been shown to achieve effective concentrations in the brain. The results of the study showed that daily treatment with ceftriaxone (200mg/kg i.p. 7d) delayed loss of muscle strength and body weight, and improved overall survival.

Ceftriaxone has also been shown to combat excitotoxicity in models of Huntington's disease and multiple sclerosis (Miller 08, Melzer 08). Subsequent studies have tested ceftriaxone in other models of excitotoxicity. Chu, et al. (2007) tested ceftriaxone in a more acute model of injury. They found that rats pretreated with five daily injections of ceftriaxone (200mg/kg ip) exhibited reduced infarct volume following ischemic induction by middle cerebral artery occlusion. However, no protective effect was observed when the drug was administered following injury.

In unpublished reports, we examined the effect of ceftriaxone treatment using the lateral fluid percussion model of TBI. We found that daily injections of the drug (200mg/kg b.w.) did not significantly increase the expression of GLT-1 protein or mRNA. Additionally, we found that ceftriaxone pretreatment did not attenuate injury-induced reductions in GLT-1 expression. Further study is required to determine if ceftriaxone can be therapeutic for the treatment of TBI.

Materials and Methods

Drug treatment

Male Sprague-Dawley rats (275-300g bw) were provided with food and water ad libitum. At the beginning of testing, they were given daily intraperitoneal injections of ceftriaxone (200mg/kg bw) for 1,3,5,7 days. Ceftriaxone treatment had no effect on body weight.

Lateral Fluid Percussion brain injury

The lateral fluid percussion injury (FPI) model was used as previously described (Yao 05). The FPI model has been extensively described and is a reliable method of administering injuries that are consistent with regards to location and severity. To induce injury, the rats were anesthetized with 0.5-1% isofluorane in 100%O_2 and immobilized in a stereotaxic instrument. A small incision was made at the top of the head, exposing the skull. A small hole (5mm) was drilled in the skull over the right cerebral hemisphere, 3mm posterior to Bregma and 2mm lateral to midline, exposing the dura mater. A tube was secured over the exposed brain and attached to a cylindrical saline reservoir. A pendulum was dropped onto the reservoir generating a fluid pulse of 2.5 atm onto the brain. Sham operated animals received the surgical treatment without the injury. At the appropriate experiment time point (1,3,5,7days post-injury), the animals are sacrificed by decapitation and tissue is dissected from the contralateral cortex and hippocampus, and the ispilateral cortex and hippocampus, and stored in liquid nitrogen for future analysis.

Western blotting

Western blotting was used to determine protein concentrations. Frozen brain tissue (30-40mg) was ground with mortal and pestle and sonicated in lysis buffer containing 1X phosphate-buffered saline, 0.1% Nonidet-P40, 0.1% sodium dodecyl sulfate, 5 g/L sodium deoxycholic acid, and protease inhibitors (Complete Protease Inhibitor Cocktail Tablets, Boehringer-Mannheim, Santa Maria, CA, USA). Protein was separated from tissue debris by centrifugation and total protein concentration was assessed using a Protein Assay Kit (Bio-Rad, San Diego, CA, USA). Samples 30μg of total protein were denatured for 5min at 100°C and separated by electrophoresis for 4h at 70V. The samples were then transferred to nitrocellulose membranes and probed with anti-GLT-1 antibodies (Santa Cruz Biotechnologies Inc, Santa Cruz, CA; BD Biosciences, San Jose, CA; Abcam, Inc, Cambridge, MA). After washing, the membranes were blotted with secondary antibodies conjugated to horseradish peroxidase, and treated with a chemiluminescent substrate (Pierce, Rockford, IL, USA). The bands were quantified using a Fuji Film LAS-1000 camera and LAS-1000plus software. The membrane was then stripped and reprobed with antibodies to β-actin to determine protein loading.

Quantitative real-time PCR

Quantitative real-time PCR was used to determine mRNA concentrations. Frozen brain tissue (30-40μg) was ground with mortal and pestle and sonicated in a denaturing buffer. Total RNA was extracted using the Totally RNA Kit (Ambion, Austin, TX, USA) and treated with Dnase I to remove genomic DNA. cDNA templates were synthesized by RT-PCR (42°C for 15 min, 99° for 5min, 5°C for 5min).

In quantitative real-time PCR, 5μl of cDNA template (three replicates per sample) was combined with primers designed for GLT-1 and loaded onto 96-well plates analyzed using the GeneAmp 7300 Sequence Detection System machine (Applied Biosystems). Primers for 18S rRNA were used as endogenous controls for total RNA concentration.

Results

Glutamate transporter-1 expression following TBI.

Male Sprague-Dawley rats (n=6) were administered injuries by FPI or subjected to sham surgery. At 6h, and 1, 3, 7d the animals were sacrificed and brain tissue was analyzed for GLT-1 expression. Western blotting showed that (Figure 1), in the injured cortex, GLT-1 protein expression was significantly decreased 3 days following injury (36% of control) and remains decreased at seven days post injury (57%). In the injured hippocampus, GLT-1 protein expression was significantly increased at 6h (140%), but reduced at 3 days (73%).

GLT-1 mRNA expression was determined using qRT-PCR (Figure 2). In the cortex, GLT-1 mRNA was increased (133%) at 6h and decreased (67%) at 1d post injury. In the hippocampus, GLT-1 mRNA expression increased (129%) at 6h and returned to control levels at 1d. No changes in GLT-1 protein or mRNA expression was detected in the uninjured hemisphere. These findings are consistent with previous reports of GLT-1 expression in animal models of TBI.

Glutamate transporter-1 expression following ceftriaxone treatment

To measure the effect of the drug treatment, the rats (n=6) were given daily intraperitoneal injections of ceftriaxone (200mg/kg b.w.) for 1, 3, 5 and 7 days. Twenty four hours after the final injection, the animals were sacrificed and brain tissue was analyzed for GLT-1 expression. The results show that GLT-1 protein and mRNA expression remained unchanged in the cortex and hippocampus following 1, 3, 5, or 7 days of ceftriaxone treatment (Figures 3 and 4). Additional studies determined that ceftriaxone had no effect on GLT-1 expression when administered at a lower dose (100mg/kg b.w.) for 3 and 5 days.

The effect of ceftriaxone pre-treatment on TBI.

To determine whether drug treatment could attenuate TBI-induced alterations in GLT-1, animals were given 7 daily injections with ceftriaxone (200mg/kg b.w.) and then subjected to injury or sham. The data indicate that drug pre-treatment has no effect on the expression of GLT-1 protein in the cortex or hippocampus following TBI (Figure 5 and 6). Additional studies determined that ceftriaxone had no effect when given for 3 and 5 days prior to injury.

Discussion

Because ceftriaxone had no effect on GLT-1 expression, its neuroprotective potential was not assessed. Other studies have shown that ceftriaxone can be therapeutic irrespective of its effects on GLT-1 expression (Lipski 07, Mineur 07). β-lactam antibiotics induce heat shock protein expression (Romano 04) and can act as metal chelators (Ji 05), possibly accounting for their neuroprotection and effect on GLT-1 expression.

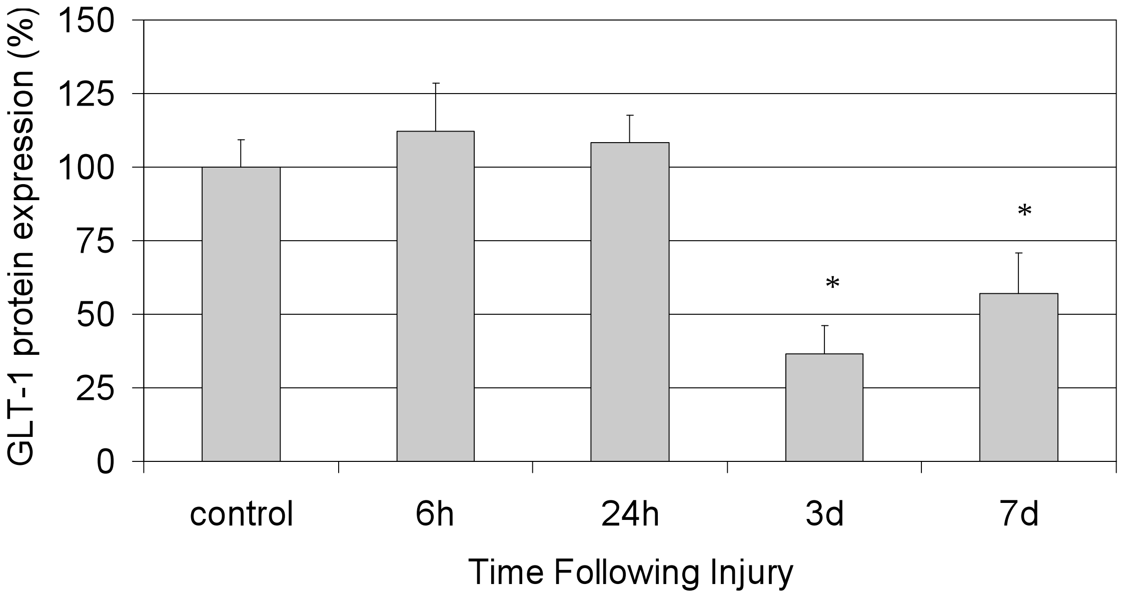
Furthermore, evidence suggests that an increase in GLT-1 expression may not be a viable treatment strategy in the context of TBI. Phillis, et al. (2000) found that glutamate transport was reversed in the rat cortex under ischemic conditions. Therefore, an increase in transporter expression would lead to an increase of glutamate in the extracellular space, further exacerbating the excitotoxic burden. Additionally, various splice variants of GLT-1 have been described (Sullivan 04), and Yi, et al. (2005) have asserted that altered expression of GLT-1v is more important in the pathological setting of the injured brain. Continued research into the expression and action of GLT-1 and other transporters is required to develop new drugs and strategies for the targeting of glutamate transport for the treatment of excitotoxicity.

Conclusion

Despite extensive research and numerous clinical trials, no therapy has proven beneficial in the treatment of TBI. Although pharmacological strategies targeting excitotoxicity have proven ineffective, various factors must be taken into account when considering these results. Because TBI causes primary injuries that cannot be prevented, the search for a treatment that can completely restore normal brain function, or a so-called "magic bullet," may be impractical. In addition, due to the nature of brain injuries, there is bound to be a great deal of variability when assessing outcome. Injuries can differ with regard to severity, site, and type of injury. Thus, the current standards for assessing neurological outcome may not be adequate. It may prove useful to determine whether a drug targeting excitotoxic cell death alleviates one specific symptom of brain injury (headache frequency, depression, or memory deficiency.)

Because brain damage following TBI is a very complex process, an effective treatment may require the action of more than one drug. A blood glutamate scavenging drug may protect against the acute excitotoxicity immediately following injury, but would be rendered ineffective when the blood-brain barrier is restored. NMDA receptor antagonists can interrupt the important physiological responses when given immediately after injury, but could potentially combat "slow excitotoxicity" and the later consequences of TBI. Additionally, actions of certain drugs could trigger compensatory processes that lead to the same or worse outcome. Thus, co-treatment with two or more drugs that target different sources of excitotoxicity, or different sources of secondary injuries (i.e. excitotoxicity, inflammation and edema), may prove more effective. Continued research is needed to develop new therapies for the millions who suffer the consequences of a TBI.

CORTEX



HIPPOCAMPUS

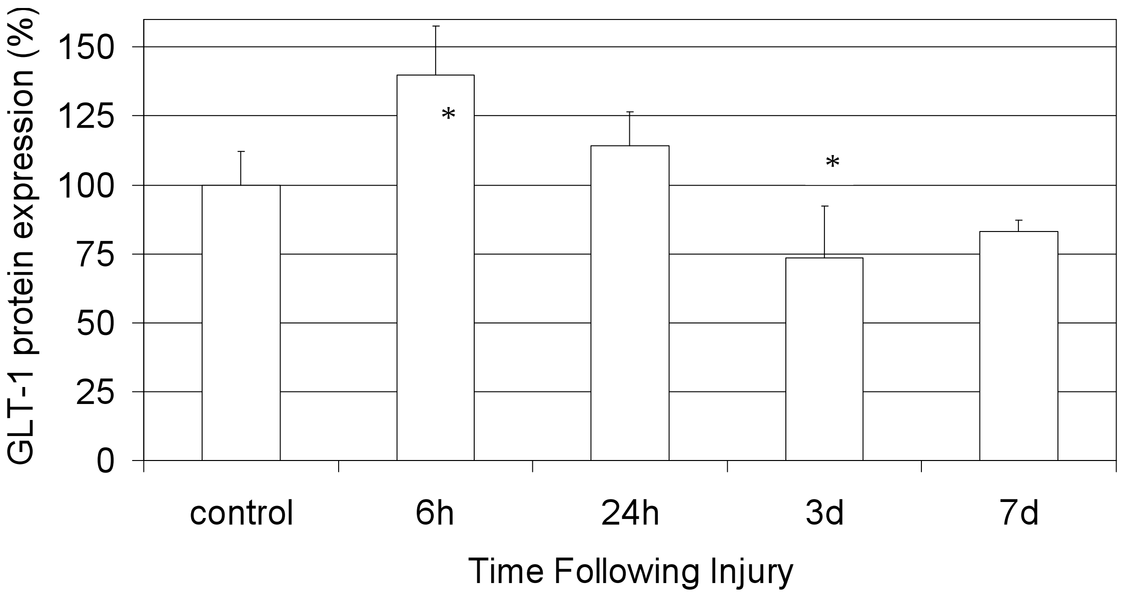
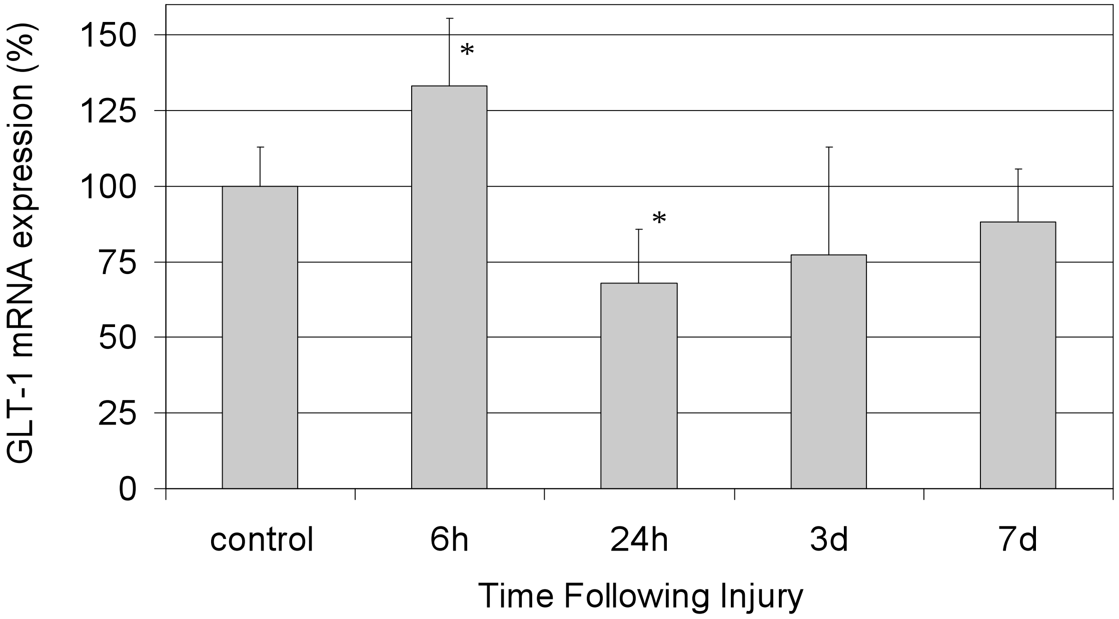


Figure 1: GLT-1 protein expression following TBI. GLT-1 protein expression in the cortex (grey) and hippocampus (white) following TBI. ($p < 0.001$)

CORTEX



HIPPOCAMPUS

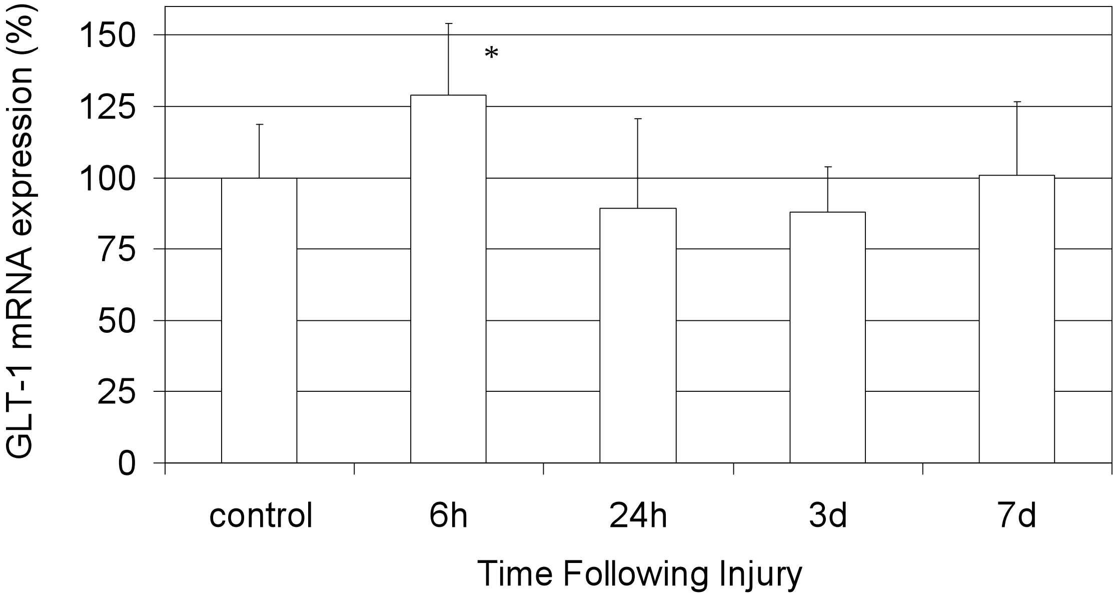
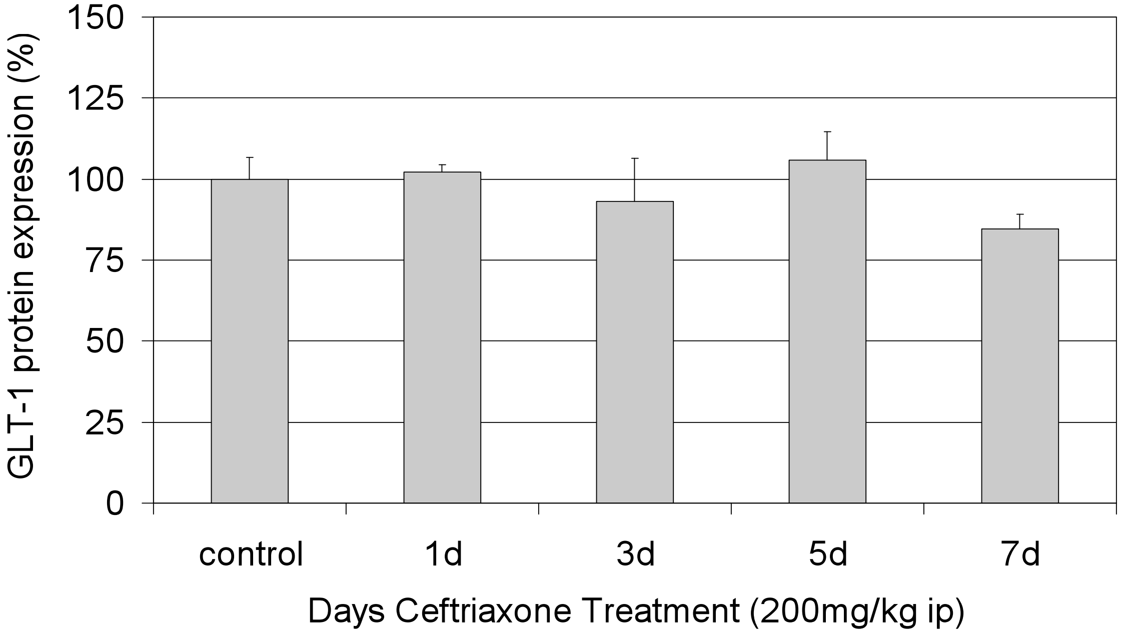


Figure 2: GLT-1 mRNA expression following TBI. GLT-1 mRNA expression in the cortex (grey) and hippocampus (white) following TBI. ($p<0.01$)

CORTEX



HIPPOCAMPUS

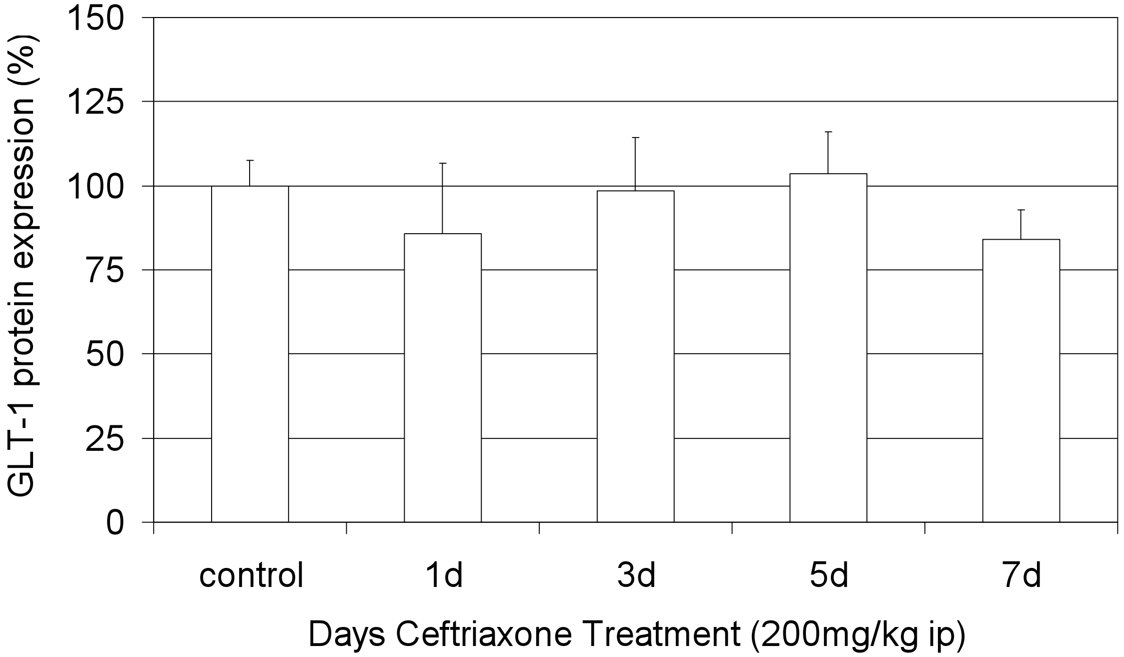
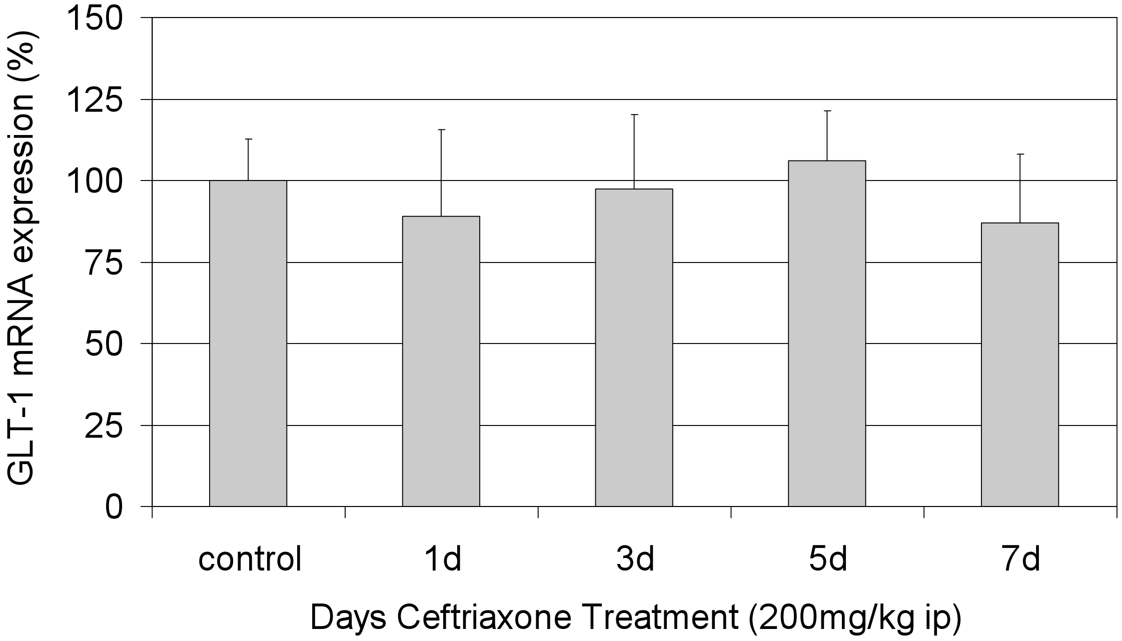


Figure 3: GLT-1 protein expression following ceftriaxone treatment. GLT-1 protein expression in the cortex (grey) and hippocampus (white) following ceftriaxone treatment.

CORTEX



HIPPOCAMPUS

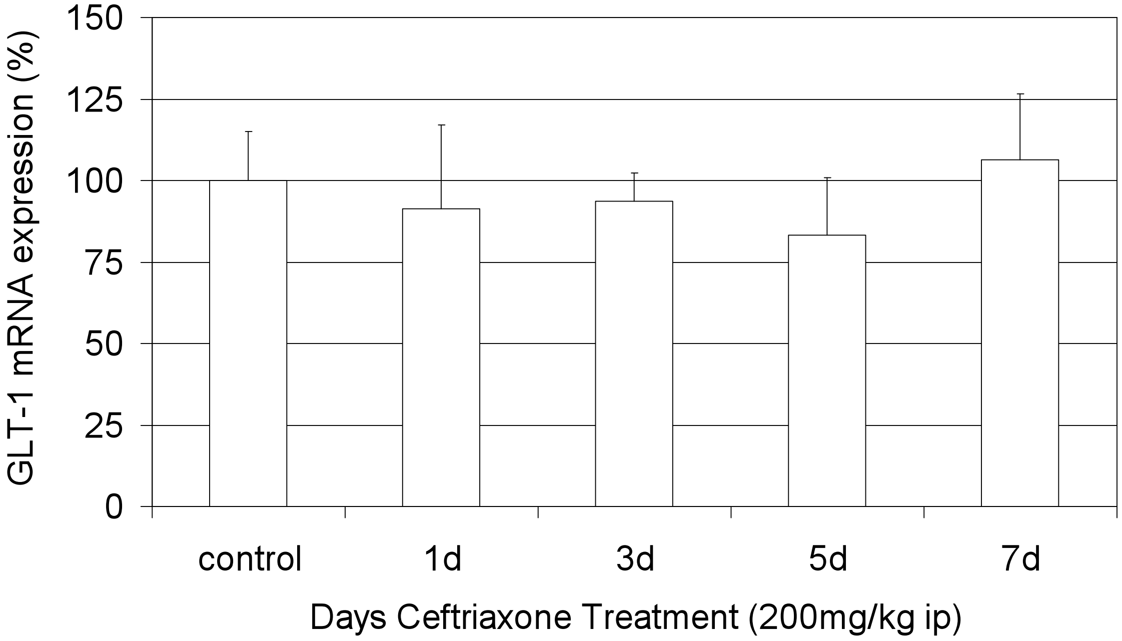


Figure 4: GLT-1 mRNA expression following ceftriaxone treatment. GLT-1 mRNA expression in the cortex (grey) and hippocampus (white) following ceftriaxone treatment.

CORTEX

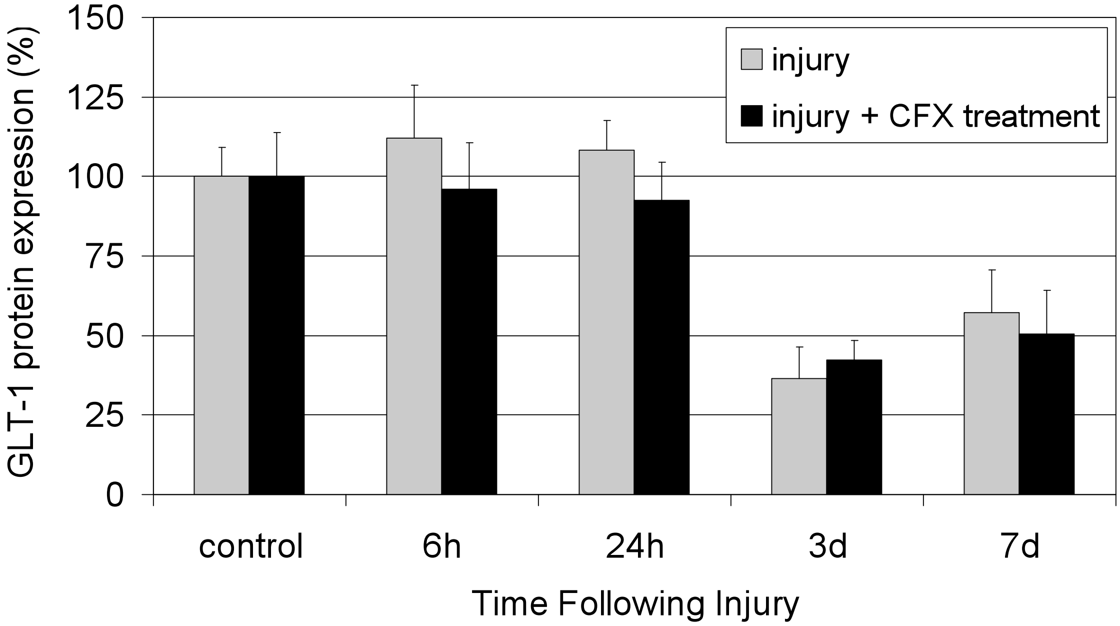


Figure 5: Effect of ceftriaxone on TBI. GLT-1 protein expression in the cortex following TBI (grey) and following TBI with ceftriaxone pre-treatment for 7d (black).

HIPPOCAMPUS

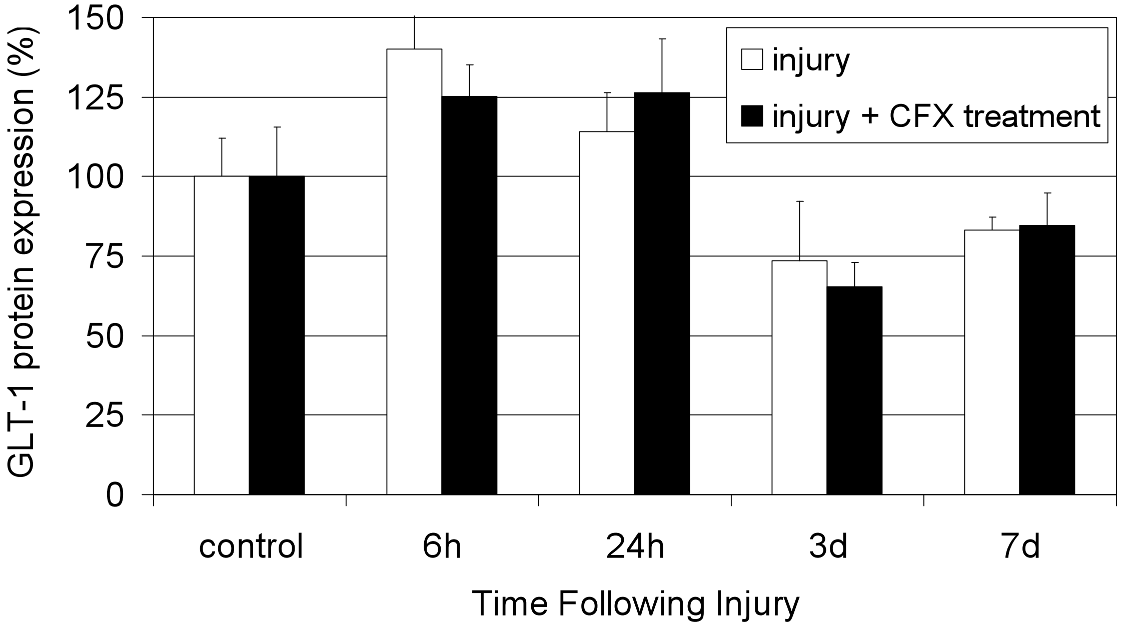


Figure 6: Effect of ceftriaxone on TBI. GLT-1 protein expression in the hippocampus following TBI (white) and following TBI with ceftriaxone pre-treatment for 7d (black).

References

Agrawal A, Timothy J, Pandit L, Manju M. Post-traumatic epilepsy: an overview. Clin Neurol Neurosurg. 2006 Jul;108(5):433-9.

Albensi BC, Janigro D. Traumatic brain injury and its effects on synaptic plasticity. Brain Inj. 2003 Aug;17(8):653-63.

Albin RL, Greenamyre JT. Alternative excitotoxic hypotheses. Neurology. 1992 Apr;42(4):733-8.

Almeida CG, Tampellini D, Takahashi RH, Greengard P, Lin MT, Snyder EM, Gouras GK. Beta-amyloid accumulation in APP mutant neurons reduces PSD-95 and GluR1 in synapses. Neurobiol Dis. 2005 Nov;20(2):187-98.

Arriza JL, Eliasof S, Kavanaugh MP, Amara SG. Excitatory amino acid transporter 5, a retinal glutamate transporter coupled to a chloride conductance.
Proc Natl Acad Sci U S A. 1997 Apr 15;94(8):4155-60

Atlante A, Gagliardi S, Marra E, Calissano P, Passarella S. Glutamate neurotoxicity in rat cerebellar granule cells involves cytochrome c release from mitochondria and mitochondrial shuttle impairment. J Neurochem. 1999 Jul;73(1):237-46.

Bailey I, Bell A, Gray J, Gullan R, Heiskanan O, Marks PV, Marsh H, Mendelow DA, Murray G, Ohman J, et al. A trial of the effect of nimodipine on outcome after head injury. Acta Neurochir (Wien). 1991;110(3-4):97-105.

Baker AJ, Moulton RJ, MacMillan VH, Shedden PM. Excitatory amino acids in cerebrospinal fluid following traumatic brain injury in humans. J Neurosurg. 1993 Sep;79(3):369-72.

Bliss TV, Gardner-Medwin AR. Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. J Physiol. 1973 Jul;232(2):357-74.

Bliss TV, Collingridge GL. A synaptic model of memory: long-term potentiation in the hippocampus. Nature. 1993 Jan 7;361(6407):31-9.

Blissitt PA. Care of the critically ill patient with penetrating head injury. Crit Care Nurs Clin North Am. 2006 Sep;18(3):321-32.

Bolay H, Reuter U, Dunn AK, Huang Z, Boas DA, Moskowitz MA. Intrinsic brain activity triggers trigeminal meningeal afferents in a migraine model. Nat Med. 2002 Feb;8(2):136-42.

Bower JH, Maraganore DM, Peterson BJ, McDonnell SK, Ahlskog JE, Rocca WA. Head trauma preceding PD: a case-control study. Neurology. 2003 May 27;60(10):1610-5.

Brown SJ, Fann JR, Grant I. Postconcussional disorder: time to acknowledge a common source of neurobehavioral morbidity. J Neuropsychiatry Clin Neurosci. 1994 Winter;6(1):15-22.

Bullock R, Zauner A, Woodward JJ, Myseros J, Choi SC, Ward JD, Marmarou A, Young HF. Factors affecting excitatory amino acid release following severe human head injury. J Neurosurg. 1998 Oct;89(4):507-18.

Chourbaji S, Vogt MA, Fumagalli F, Sohr R, Frasca A, Brandwein C, Hörtnagl H, Riva MA, Sprengel R, Gass P. AMPA receptor subunit 1 (GluR-A) knockout mice model the glutamate hypothesis of depression. FASEB J. 2008 Sep;22(9):3129-34.

Chu K, Lee ST, Sinn DI, Ko SY, Kim EH, Kim JM, Kim SJ, Park DK, Jung KH, Song EC, Lee SK, Kim M, Roh JK. Pharmacological Induction of Ischemic Tolerance by Glutamate Transporter-1 (EAAT2) Upregulation. Stroke. 2007 Jan;38(1):177-82.

Clausen T, Bullock R. Medical treatment and neuroprotection in traumatic brain injury. Curr Pharm Des. 2001 Oct;7(15):1517-32.

Colbourne F, Grooms SY, Zukin RS, Buchan AM, Bennett MV. Hypothermia rescues hippocampal CA1 neurons and attenuates down-regulation of the AMPA receptor GluR2 subunit after forebrain ischemia. Proc Natl Acad Sci U S A. 2003 Mar 4;100(5):2906-10.

Coles JP, Fryer TD, Smielewski P, Rice K, Clark JC, Pickard JD, Menon DK. Defining ischemic burden after traumatic brain injury using ^{15}O PET imaging of cerebral physiology. J Cereb Blood Flow Metab. 2004 Feb;24(2):191-201.

Cotman CW, Monaghan DT. Anatomical organization of excitatory amino acid receptors and their properties. Adv Exp Med Biol. 1986;203:237-52.

Danbolt NC. Glutamate uptake. Prog Neurobiol. 2001 Sep;65(1):1-105.

Danbolt NC, Storm-Mathisen J, Kanner BI. An [Na^{+} $^{+}$ K^{+}]coupled L-glutamate transporter purified from rat brain is located in glial cell processes. Neuroscience. 1992 Nov;51(2):295-310

Dehnes Y, Chaudhry FA, Ullensvang K, Lehre KP, Storm-Mathisen J, Danbolt NC. The glutamate transporter EAAT4 in rat cerebellar Purkinje cells: a glutamate-gated chloride channel concentrated near the synapse in parts of the dendritic membrane facing astroglia. J Neurosci. 1998 May 15;18(10):3606-19.

Doble A. The role of excitotoxicity in neurodegenerative disease: implications for therapy. Pharmacol Ther. 1999 Mar;81(3):163-221.

Faden AI, Demediuk P, Panter SS, Vink R. The role of excitatory amino acids and NMDA receptors in traumatic brain injury. Science. 1989 May 9;244(4906):798-800.

Foster AC, Fagg GE. Acidic amino acid binding sites in mammalian neuronal membranes: their characteristics and relationship to synaptic receptors. Brain Res. 1984 May;319(2):103-64.

Gascón S, Sobrado M, Roda JM, Rodríguez-Peña A, Díaz-Guerra M. Excitotoxicity and focal cerebral ischemia induce truncation of the NR2A and NR2B subunits of the NMDA receptor and cleavage of the scaffolding protein PSD-95. Mol Psychiatry. 2008 Jan;13(1):99-114..

Geddes-Klein DM, Serbest G, Mesfin MN, Cohen AS, Meaney DF. Pharmacologically induced calcium oscillations protect neurons from increases in cytosolic calcium after trauma. J Neurochem. 2006 Apr;97(2):462-74.

Goto Y, Niidome T, Akaike A, Kihara T, Sugimoto H. Amyloid beta-peptide preconditioning reduces glutamate-induced neurotoxicity by promoting endocytosis of NMDA receptor. Biochem Biophys Res Commun. 2006 Dec 8;351(1):259-65.

Hall RC, Hall RC, Chapman MJ. Definition, diagnosis, and forensic implications of postconcussional syndrome. Psychosomatics. 2005 May-Jun;46(3):195-202.

Ikonomidou C, Turski L. Why did NMDA receptor antagonists fail clinical trials for stroke and traumaticbrain injury? Lancet Neurol. 2002 Oct;1(6):383-6.

Izquierdo I, Medina JH. Memory formation: the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. Neurobiol Learn Mem. 1997 Nov;68(3):285-316.

Janjua NA, Mori A, Hiramatsu M. Increased aspartic acid release from the iron induced epileptogenic focus. Epilepsy Res 1990;6:215–20.

Ji HF, Shen L, Zhang HY. Beta-lactam antibiotics are multipotent agents to combat neurological diseases. Biochem Biophys Res Commun. 2005 Aug 5;333(3):661-3.

Kakarieka A, Braakman R, Schakel EH. Clinical significance of the finding of subarachnoid blood on CT scan after head injury. Acta Neurochir (Wien). 1994;129(1-2):1-5.

Kelly DF, Martin NA, Kordestani R, Counelis G, Hovda DA, Bergsneider M,McBride DQ, Shalmon E, Herman D, Becker DP. Cerebral blood flow as a predictor of outcome following traumatic brain injury. J Neurosurg. 1997 Apr;86(4):633-41.

Khoshyomn S, Tranmer BI. Diagnosis and management of pediatric closed head injury. Semin Pediatr Surg. 2004 May;13(2):80-6.

Koizumi H, Fujisawa H, Ito H, Maekawa T, Di X, Bullock R. Effects of mild hypothermia on cerebral blood flow-independent changes in cortical extracellular levels of amino acids following contusion trauma in the rat. Brain Res. 1997 Feb 7;747(2):304-12.

Kraus JF, Fife D, Ramstein K, Conroy C, Cox P. The relationship of family income to the incidence, external causes, and outcomes of serious brain injury, San Diego County, California. Am J Public Health. 1986 Nov;76(11):1345-7.

Kroppenstedt SN, Schneider GH, Thomale UW, Unterberg AW. Protective effects of aptiganel HCl (Cerestat) following controlled cortical impact injury in the rat. J Neurotrauma. 1998 Mar;15(3):191-7

Langlois JA, Rutland-Brown W, Thomas KE. Traumatic brain injury in the United States: emergency department visits, hospitalizations, and deaths. Atlanta (GA): Centers for Disease Control and Prevention, Nation Center for Injury Prevention and Control; 2004.

Lipski J, Wan CK, Bai JZ, Pi R, Li D, Donnelly D. Neuroprotective potential of ceftriaxone in in vitro models of stroke. Neuroscience. 2007 May 11;146(2):617-29.

Lucas SM, Rothwell NJ, Gibson RM. The role of inflammation in CNS injury and disease. Br J Pharmacol. 2006 Jan;147 Suppl 1:S232-40.

Lucetti C, Del Dotto P, Gambaccini G, Dell' Agnello G, Bernardini S, Rossi G, Murri L, Bonuccelli U. IV amantadine improves chorea in Huntington's disease: an acute randomized,controlled study. Neurology. 2003 Jun 24;60(12):1995-7

Marklund N, Bakshi A, Castelbuono DJ, Conte V, McIntosh TK. Evaluation of pharmacological treatment strategies in traumatic brain injury. Curr Pharm Des. 2006;12(13):1645-80.

McIntosh TK, Juhler M, Wieloch T. Novel pharmacologic strategies in the treatment of experimental traumatic brain injury: 1998. J Neurotrauma. 1998 Oct;15(10):731-69.

Melzer N, Meuth SG, Torres-Salazar D, Bittner S, Zozulya AL, Weidenfeller C, Kotsiari A, Stangel M, Fahlke C, Wiendl H. A beta-lactam antibiotic dampens excitotoxic inflammatory CNS damage in a mouse model of multiple sclerosis. PLoS ONE. 2008 Sep 5;3(9):e3149.

Miller BR, Dorner JL, Shou M, Sari Y, Barton SJ, Sengelaub DR, Kennedy RT, Rebec GV. Up-regulation of GLT1 expression increases glutamate uptake and attenuates the Huntington's disease phenotype in the R6/2 mouse. Neuroscience. 2008 Apr 22;153(1):329-37.

Mineur YS, Picciotto MR, Sanacora G. Antidepressant-like effects of ceftriaxone in male C57BL/6J mice. Biol Psychiatry. 2007 Jan 15;61(2):250-2.

Morris GF, Bullock R, Marshall SB, Marmarou A, Maas A, Marshall LF. Failure of the competitive N-methyl-D-aspartate antagonist Selfotel (CGS 19755) in the treatment of severe head injury: results of two phase III clinical trials. The Selfotel Investigators. J Neurosurg. 1999 Nov;91(5):737-43

Moskowitz MA, Nozaki K, Kraig RP. Neocortical spreading depression provokes the expression of c-fos protein-like immunoreactivity within trigeminal nucleus caudalis via trigeminovascular mechanisms. J Neurosci. 1993 Mar;13(3):1167-77.

Mukhin A, Fan L, Faden AI. Activation of metabotropic glutamate receptor subtype mGluR1 contributes to post-traumatic neuronal injury. J Neurosci. 1996 Oct 1;16(19):6012-20.

Murray GD, Teasdale GM, Schmitz H. Nimodipine in traumatic subarachnoid haemorrhage: a re-analysis of the HIT I and HIT II trials. Acta Neurochir (Wien). 1996;138(10):1163-7.

Narayan RK, Michel ME, Ansell B, Baethmann A, Biegon A, Bracken MB, Bullock MR, Choi SC, Clifton GL, Contant CF, Coplin WM, Dietrich WD, Ghajar J, Grady SM, Grossman RG, Hall ED, Heetderks W, Hovda DA, Jallo J, Katz RL, Knoller N, Kochanek PM, Maas AI, Majde J, Marion DW, Marmarou A, Marshall LF, McIntosh TK, Miller E, Mohberg N, Muizelaar JP, Pitts LH, Quinn P, Riesenfeld G, Robertson CS, Strauss KI, Teasdale G, Temkin N, Tuma R, Wade C, Walker MD, Weinrich M, Whyte J, Wilberger J, Young AB, Yurkewicz L. Clinical trials in head injury. J Neurotrauma. 2002 May;19(5):503-57.

Olney JW. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate.Science. 1969 May 9;164(880):719-21.

Olsson A, Csajbok L, Ost M, Höglund K, Nylén K, Rosengren L, Nellgård B, Blennow K. Marked increase of beta-amyloid(1-42) and amyloid precursor protein in ventricular cerebrospinal fluid after severe traumatic brain injury. J Neurol. 2004 Jul;251(7):870-6.

Osteen CL, Giza CC, Hovda DA. Injury-induced alterations in N-methyl-D-aspartate receptor subunit composition contribute to prolonged 45calcium accumulation following lateral fluid percussion. Neuroscience. 2004;128(2):305-22.

Panikashvili D, Shein NA, Mechoulam R, Trembovler V, Kohen R, Alexandrovich A, Shohami E. The endocannabinoid 2-AG protects the blood-brain barrier after closed head injury and inhibits mRNA expression of proinflammatory cytokines. Neurobiol Dis. 2006 May;22(2):257-64.

Peeters M, Gunthorpe MJ, Strijbos PJ, Goldsmith P, Upton N, James MF. Effects of pan- and subtype-selective N-methyl-D-aspartate receptor antagonists on cortical spreading depression in the rat: therapeutic potential for migraine. J Pharmacol Exp Ther. 2007 May;321(2):564-72.

Phillis JW, Ren J, O'Regan MH. Transporter reversal as a mechanism of glutamate release from the ischemic rat cerebral cortex: studies with DL-threo-beta-benzyloxyaspartate. Brain Res. 2000 Oct 13;880(1-2):224. No abstract available.

Pike BR, Zhao X, Newcomb JK, Posmantur RM, Wang KK, Hayes RL. Regional calpain and caspase-3 proteolysis of alpha-spectrin after traumatic brain injury. Neuroreport. 1998 Aug 3;9(11):2437-42

Rao VL, Başkaya MK, Doğan A, Rothstein JD, Dempsey RJ. Traumatic brain injury down-regulates glial glutamate transporter (GLT-1 and GLAST) proteins in rat brain. J Neurochem. 1998 May;70(5):2020-7.

Rao VL, Dogan A, Bowen KK, Todd KG, Dempsey RJ. Antisense knockdown of the glial glutamate transporter GLT-1 exacerbates hippocampal neuronal damage following traumatic injury to rat brain. Eur J Neurosci. 2001 Jan;13(1):119-28.

Rao VL, Dogan A, Todd KG, Bowen KK, Dempsey RJ. Neuroprotection by memantine, a non-competitive NMDA receptor antagonist after traumatic brain injury in rats. Brain Res. 2001 Aug 17;911(1):96-100.

Rao V, Lyketsos CG. Psychiatric aspects of traumatic brain injury. Psychiatr Clin North Am. 2002 Mar;25(1):43-69.

Rao V, Lyketsos C. Neuropsychiatric sequelae of traumatic brain injury. Psychosomatics. 2000 Mar-Apr;41(2):95-103.

Reisberg B, Doody R, Stöffler A, Schmitt F, Ferris S, Möbius HJ; Memantine Study Group. Memantine in moderate-to-severe Alzheimer's disease. N Engl J Med. 2003 Apr 3;348(14):1333-41.

Richens A, Mawer G, Crawford P, Harrison B. A placebo-controlled, double-blind cross-over trial of adjunctive one month remacemide hydrochloride treatment in patients with refractory epilepsy. Seizure. 2000 Dec;9(8):537-43.

Rogatsky GG, Sonn J, Kamenir Y, Zarchin N, Mayevsky A. Relationship between intracranial pressure and cortical spreading depression following fluid percussion brain injury in rats. J Neurotrauma. 2003 Dec;20(12):1315-25.

Romano CC, Benedetto N, Catania MR, Rizzo A, Gallè F, Losi E, Hasty DL, Rossano F. Commonly used antibiotics induce expression of Hsp 27 and Hsp 60 and protect human lymphocytes from apoptosis. Int Immunopharmacol. 2004 Aug;4(8):1067-73.

Roselli F, Tirard M, Lu J, Hutzler P, Lamberti P, Livrea P, Morabito M, Almeida OF. Soluble beta-amyloid1-40 induces NMDA-dependent degradation of postsynaptic density-95 at glutamatergic synapses. J Neurosci. 2005 Nov 30;25(48):11061-70.

Roth P, Farls K. Pathophysiology of traumatic brain injury. Crit Care Nurs Q. 2000 Nov;23(3):14-25; quiz 65.

Rothman SM. The neurotoxicity of excitatory amino acids is produced by passive chloride influx. J Neurosci. 1985 Jun;5(6):1483-9.

Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE, Jin L, Dykes Hoberg M, Vidensky S, Chung DS, Toan SV, Bruijn LI, Su ZZ, Gupta P, Fisher PB. Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. Nature. 2005 Jan 6;433(7021):73-7.

Rothstein JD, Van Kammen M, Levey AI, Martin LJ, Kuncl RW. Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. Ann Neurol. 1995 Jul;38(1):73-84.

Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, Kanai Y, Hediger MA, Wang Y, Schielke JP, Welty DF. Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron. 1996 Mar;16(3):675-86.

Rothstein JD, Martin L, Levey AI, Dykes-Hoberg M, Jin L, Wu D, Nash N, Kuncl RW. Localization of neuronal and glial glutamate transporters. Neuron. 1994 Sep;13(3):713-25.

Rutland-Brown W, Langlois JA, Thomas KE, Xi YL. Incidence of traumatic brain injury in the United States, 2003. J Head Trauma Rehabil. 2006 Nov-Dec;21(6):544-8.

Ruppel RA, Kochanek PM, Adelson PD, Rose ME, Wisniewski SR, Bell MJ, Clark RS, Marion DW, Graham SH. Excitatory amino acid concentrations in ventricular cerebrospinal fluid after severe traumatic brain injury in infants and children: the role of child abuse. J Pediatr. 2001 Jan;138(1):18-25.

Sanacora G, Gueorguieva R, Epperson CN, Wu YT, Appel M, Rothman DL, Krystal JH, Mason GF. Subtype-specific alterations of gamma-aminobutyric acid and glutamate in patients with major depression. Arch Gen Psychiatry. 2004 Jul;61(7):705-13.

Sepkuty JP, Cohen AS, Eccles C, Rafiq A, Behar K, Ganel R, Coulter DA, Rothstein JD. A neuronal glutamate transporter contributes to neurotransmitter GABA synthesis and epilepsy. J Neurosci. 2002 Aug 1;22(15):6372-9.

Shen M, Piser TM, Seybold VS, Thayer SA. Cannabinoid receptor agonists inhibit glutamatergic synaptic transmission in rat hippocampal cultures. J Neurosci. 1996 Jul 15;16(14):4322-34.

Shen J, Rothman DL, Behar KL, Xu S. Determination of the glutamate-glutamine cycling flux using two-compartment dynamic metabolic modeling is sensitive to astroglial dilution. J Cereb Blood Flow Metab. 2008 Sep 3.

Smith DH, Chen XH, Iwata A, Graham DI. Amyloid beta accumulation in axons after traumatic brain injury in humans. J Neurosurg. 2003 May;98(5):1072-7.

Solomon S. Posttraumatic headache. Med Clin North Am. 2001 Jul;85(4):987-96, vii-viii.

Stiefel MF, Tomita Y, Marmarou A. Secondary ischemia impairing the restoration of ion homeostasis following traumatic brain injury. J Neurosurg. 2005 Oct;103(4):707-14.

Sullivan R, Rauen T, Fischer F, Wiessner M, Grewer C, Bicho A, Pow DV. Cloning, transport properties, and differential localization of two splice variants of GLT-1 in the rat CNS: implications for CNS glutamate homeostasis. Glia. 2004 Jan 15;45(2):155-69.

Szczygielski J, Mautes A, Steudel WI, Falkai P, Bayer TA, Wirths O. Traumatic brain injury: cause or risk of Alzheimer's disease? A review of experimental studies. J Neural Transm. 2005 Nov;112(11):1547-64. Epub 2005 Jun 15.

Thurman DJ, Alverson C, Dunn KA, Guerrero J, Sniezek JE. Traumatic brain injury in the United States: A public health perspective. J Head Trauma Rehabil. 1999 Dec;14(6):602-15.

Teasdale G, Jennett B. Assessment of coma and impaired consciousness. A practical scale. Lancet. 1974 Jul 13;2(7872):81-4.

Teasdale G, Bailey I, Bell A, Gray J, Gullan R, Heiskanan U, Marks PV, Marsh H, Mendelow AD, Murray G, et al. The effect of nimodipine on outcome after head injury: a prospective randomised control trial. The British/Finnish Co-operative Head Injury Trial Group. Acta Neurochir Suppl (Wien). 1990;51:315-6.

Tolias CM, Richards DA, Bowery NG, Sgouros S. Extracellular glutamate in the brains of children with severe head injuries: a pilot microdialysis study. Childs Nerv Syst. 2002 Aug;18(8):368-74.

Toms NJ, Roberts PJ, Salt TE, Staton PC. Latest eruptions in metabotropic glutamate receptors. Trends Pharmacol Sci. 1996 Dec;17(12):429-35.

Unterberg AW, Stover J, Kress B, Kiening KL. Edema and brain trauma. Neuroscience. 2004;129(4):1021-9.

von Baumgarten L, Trabold R, Thal S, Back T, Plesnila N. Role of cortical spreading depressions for secondary brain damage after traumatic brain injury in mice. J Cereb Blood Flow Metab. 2008 Jul;28(7):1353-60.

Wahl F, Renou E, Mary V, Stutzmann JM. Riluzole reduces brain lesions and improves neurological function in rats after a traumatic brain injury. Brain Res. 1997 May 9;756(1-2):247-55.

Warden D. Military TBI during the Iraq and Afghanistan wars. J Head Trauma Rehabil. 2006 Sep-Oct;21(5):398-402

Weight DG. Minor head trauma. Psychiatr Clin North Am. 1998 Sep;21(3):609-24.

Werner C, Engelhard K. Pathophysiology of traumatic brain injury. Br J Anaesth. 2007 Jul;99(1):4-9.

Willmore LJ, Sypert GW, Munson JB. Recurrent seizures induced by cortical iron injection: a model of posttraumatic epilepsy. Ann Neurol 1978;4:329–36.

Yakovlev AG, Di X, Movsesyan V, Mullins PG, Wang G, Boulares H, Zhang J, Xu M, Faden AI. Presence of DNA fragmentation and lack of neuroprotective effect in DFF45 knockout mice subjected to traumatic brain injury. Mol Med. 2001 Mar;7(3):205-16.

Yao XL, Liu J, Lee E, Ling GS, McCabe JT. Progesterone differentially regulates pro- and anti-apoptotic gene expression in cerebral cortex following traumatic brain injury in rats. J Neurotrauma. 2005 Jun;22(6):656-68.

Yi JH, Pow DV, Hazell AS. Early loss of the glutamate transporter splice-variant GLT-1v in rat cerebral cortex following lateral fluid-percussion injury. Glia. 2005 Jan 1;49(1):121-33.

Yi JH, Hoover R, McIntosh TK, Hazell AS. Early, transient increase in complexin I and complexin II in the cerebral cortex following traumatic brain injury is attenuated by N-acetylcysteine. J Neurotrauma. 2006 Jan;23(1):86-96

Yurkewicz L, Weaver J, Bullock MR, Marshall LF. The effect of the selective NMDA receptor antagonist traxoprodil in the treatment of traumatic brain injury. J Neurotrauma. 2005 Dec;22(12):1428-43.

Zarate CA Jr, Payne JL, Quiroz J, Sporn J, Denicoff KK, Luckenbaugh D, Charney DS, Manji HK. An open-label trial of riluzole in patients with treatment-resistant major depression. Am J Psychiatry. 2004 Jan;161(1):171-4.

Zhang C, Raghupathi R, Saatman KE, Smith DH, Stutzmann JM, Wahl F, McIntosh TK. Riluzole attenuates cortical lesion size, but not hippocampal neuronal loss, following traumatic brain injury in the rat. J Neurosci Res. 1998 May 1;52(3):342-9.

Zlotnik A, Gurevich B, Cherniavsky E, Tkachov S, Matuzani-Ruban A, Leon A, Shapira Y, Teichberg VI. The contribution of the blood glutamate scavenging activity of pyruvate to its neuroprotective properties in a rat model of closed head injury. Neurochem Res. 2008 Jun;33(6):1044-50.

Zlotnik A, Gurevich B, Tkachov S, Maoz I, Shapira Y, Teichberg VI. Brain neuroprotection by scavenging blood glutamate. Exp Neurol. 2007 Jan;203(1):213-20.

Zukin RS, Bennett MV. Alternatively spliced isoforms of the NMDARI receptor subunit. Trends Neurosci. 1995 Jul;18(7):306-13. Erratum in: Trends Neurosci 1995 Oct;18(10):441.

Zwienenberg M, Gong QZ, Berman RF, Muizelaar JP, Lyeth BG. The effect of groups II and III metabotropic glutamate receptor activation on neuronal injury in a rodent model of traumatic brain injury. Neurosurgery. 2001 May;48(5):1119-26.